



Brief Rapid Communications

Anti-Tumor Necrosis Factor- α Antibody Limits Heart Failure in a Transgenic Model

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Background—Tumor necrosis factor (TNF)- α has been implicated in the pathophysiology of congestive heart failure. A strain of transgenic mice (TNF1.6) with cardiac-specific overexpression of TNF- α develop congestive heart failure.

Methods and Results—To determine the effect of anti-TNF- α therapy in this model, we studied 3 groups: TNF1.6 mice treated with saline, wild-type mice treated with saline, and TNF1.6 mice treated with TNF- α neutralizing antibody (cV1q) from 6 to 12 weeks of age. We used echocardiography to compare cardiac hypertrophy, function, and catecholamine response at 12 weeks of age versus baseline (6 weeks). cV1q treatment did not limit cardiac hypertrophy, but it significantly improved basal fractional shortening and responsiveness to β -adrenergic stimulation, and it limited development of cardiac dilation.

Conclusions—Blockade of TNF- α bioactivity by antibody therapy may both preserve cardiac function and partially reverse pathological changes in congestive heart failure. (*Circulation*. 2001;104:1094-1097.)

Key Words: antibodies ■ heart failure ■ hormones

Tumor necrosis factor (TNF)- α may play a pathophysiological role in human heart failure.^{1,2} To assess the potential role of TNF- α in heart failure, we generated a transgenic model in which heart failure arises as a consequence of cardiac-specific expression of murine TNF- α .³ These mice (TNF1.6) display cardiac dilation and fibrosis, loss of cardiac function, reduction of response to β -adrenergic stimulation, increased cardiac infiltrates, enhanced expression of cytokines downstream to TNF- α , re-expression of the fetal gene program, and reduced survival.³⁻⁵ Interestingly, in this model male mice are more severely affected than are female mice.⁶

Anti-TNF- α therapies have proved successful in the treatment of inflammatory diseases, including rheumatoid arthritis and inflammatory bowel diseases. Such therapies may use fusion proteins consisting of soluble TNF receptor and immunoglobulin G (IgG)⁷ or antibodies directed against TNF- α .^{8,9} Presumably, these agents sequester TNF- α and block interaction with cellular TNF receptors, thereby blunting the biological effects of TNF- α . Previous studies have investigated the utility of receptor-fusion proteins in the treatment of animal models of heart failure.^{5,10} In the present study, we investigate the utility of a monoclonal antibody (cV1q) directed against murine TNF- α in modifying cardiac function in the TNF1.6 model of heart failure.

Methods

Characterizations of TNF1.6 mice have been reported previously.³⁻⁶ Animal use protocols were approved by the Institutional Animal

Care and Use Committee. At 6 weeks of age, all mice received echocardiographic assessment. Mice then received either sterile saline (control; both wild-type [WT] and TNF1.6 mice) or the antibody cV1q (IP 0.5 mg/mouse per week; TNF1.6 mice only) for 6 weeks. At 12 weeks of age, mice were again examined by echocardiography. The mice were then euthanized, and tissues were collected for analysis.

cV1q (Centocor) is a chimeric rat/mouse monoclonal antibody with neutralizing activity against mouse TNF- α . M-mode echocardiographic analyses (baseline and after isoproterenol challenge) were performed as previously described.⁴⁻⁶ Measurements included left ventricular diastolic dimension (LVDD), left ventricular systolic dimension (LVSD), percentage fractional shortening (%FS), and left ventricular (LV) mass index (LV:body [mg/g]). Cardiac TNF- α and interleukin (IL)-1 β levels were measured as previously described.^{3,5,6}

Results are reported as mean \pm SEM. Comparisons between sexes were performed using ANOVA with Student-Newman-Keuls post-hoc tests. Comparisons between mice analyzed at 6 weeks (baseline) and 12 weeks (end of treatment) were performed by paired *t* tests. Differences were considered significant at $P < 0.05$.

Results

Echocardiographic Studies

Between 6 and 12 weeks of age, male TNF1.6 mice showed more profound cardiac dysfunction than did female TNF1.6 mice.⁶ Age-matched mice were grouped by sex, using male TNF1.6 mice as a model of moderate heart failure and female TNF1.6 mice as a model of mild, progressive heart failure. When grouped by age and sex, 6-week-old WT male mice showed a significantly greater LVDD compared with females (males, 3.89 ± 0.05 mm; females, 3.66 ± 0.05 mm; $P < 0.05$).

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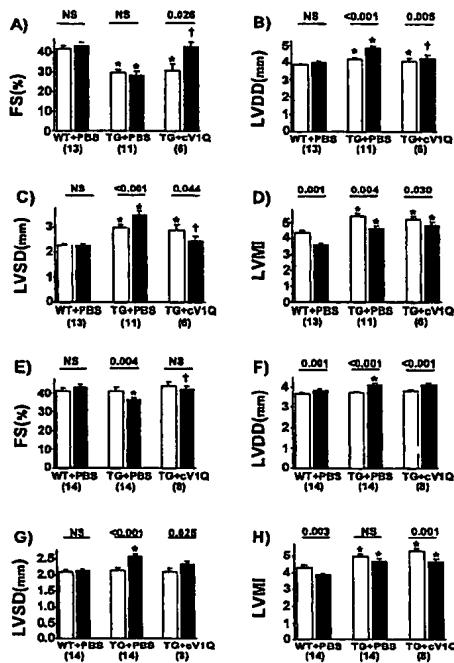


Figure 1. Echocardiographic analyses. A-D show results for male mice and E-H, female mice. Open bars represent mice at 6 weeks of age (baseline) and solid bars, 12 weeks of age (end of treatment). TG indicates TNF1.6 mice; PBS, phosphate-buffered saline; and LVMI, left ventricular mass index (mg/g body weight). Number of mice in each group is shown in parentheses. Numbers over bars indicate significance (paired *t* test) between baseline and end of treatment. **P*<0.05 (ANOVA) relative to WT mice of same sex and age; †*P*<0.05 versus TG+PBS.

However, no sex-specific differences in LVSD, %FS, or LV mass index were observed in either 6- or 12-week-old mice, nor were there sex-specific differences in LVDD measured in 12-week-old mice (data not shown). At 6 weeks of age, TNF1.6 males (but not females) showed a significantly reduced basal %FS and increased LVDD and LVSD relative to sex-matched WT mice (Figure 1A-G). Both male and female TNF1.6 mice showed a significant increase in LV mass index relative to sex-matched 6-week-old WT mice (Figure 1C and F), which was not statistically different between the sexes (data not shown).

When treated with saline for 6 weeks (control), male TNF1.6 mice retained an enhanced LV mass index and depressed %FS, whereas cardiac dilation (LVDD and LVSD) significantly increased (Figure 1A-D). Female TNF1.6 mice treated with saline for 6 weeks developed indices of heart failure, as suggested by a significantly reduced %FS and increased measures of cardiac dilation (Figure 1E, F, and H) when compared with either age-matched WT or 6-week-old TNF1.6 female mice.

Treatment with cV1q significantly preserved cardiac function and limited changes in cardiac dilation in both male and female TNF1.6 mice. Thus, %FS in cV1q-treated male mice increased significantly, was significantly higher than that of saline-treated TNF1.6 males, and was equivalent to that of WT males. Both LVDD and LVSD were significantly lower

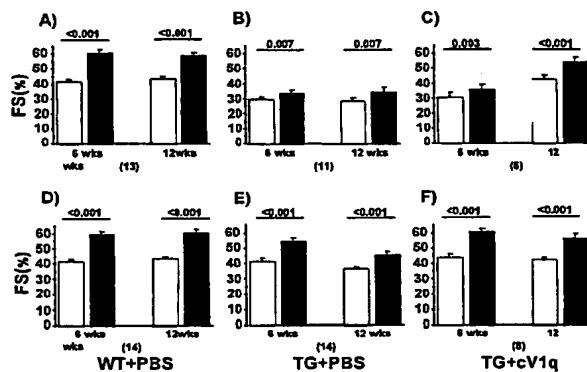


Figure 2. Assessment of response to isoproterenol. Response was assessed at baseline (6 wks) and end of treatment (12 wks). Open columns represent measurement before isoproterenol; solid columns, measurement after isoproterenol (300 ng/g body weight IP). A-C show results for male mice and D-F, female mice. A and D show WT+PBS group; B and E, TNF1.6+PBS; and C and F, TNF1.6+cV1q. Number of mice in each group is shown in parentheses. PBS indicates phosphate-buffered saline.

in cV1q-treated males than in saline-treated TNF1.6 males and were equivalent to that of WT males (Figure 1A-C). cV1q-treated females showed a %FS significantly higher than that of saline-treated TNF1.6 females and equivalent to that of age-matched WT females. Cardiac dilation did progress in TNF1.6 females treated with cV1q but was not significantly larger than in age-matched WT females treated with saline, whereas saline-treated TNF1.6 females did show significantly increased LVDD and LVSD (Figure 1E-G). Interestingly, in both male and female TNF1.6 mice, cV1q did not reduce the cardiac hypertrophy already evident at 6 weeks of age when measured by echocardiographic determination of LV mass index (Figure 1D and H) or by gravimetric measure of ventricle mass (data not shown).

Response to β -adrenergic stimulation was also improved in TNF1.6 mice treated with cV1q (Figure 2). Both 6- and 12-week-old male and female WT mice showed similar responses to isoproterenol stimulation, with a marked increase in %FS (Figure 2A and D). Consistent with the more severe heart failure of male TNF1.6, the markedly depressed basal %FS was significantly increased in response to isoproterenol to a much smaller extent in both 6- and 12-week-old male mice (Figure 2B). Female TNF1.6 mice showed a near-normal response to isoproterenol at 6 weeks of age and a significant, although reduced, response at 12 weeks of age (Figure 2E). After treatment with cV1q for 6 weeks, both male and female TNF1.6 mice displayed a significantly increased response to isoproterenol relative to saline-treated TNF1.6 mice and statistically equivalent to that observed in sex-matched saline-treated WT mice (male TNF1.6+saline, $5.6 \pm 1.65\%$ [P <0.05 relative to other males by ANOVA]; male TNF1.6+cV1q, $11.6 \pm 1.42\%$; male WT+saline, $15.6 \pm 1.48\%$; female TNF1.6+saline, $9.4 \pm 1.5\%$ [P <0.05 relative to other females by ANOVA]; female TNF1.6+cV1q, $14.3 \pm 1.87\%$; female WT+saline, $17.0 \pm 1.40\%$).

Myocardial Levels of TNF- α and IL-1 β in 12-Week-Old TNF1.6 Mice Treated With Saline or cV1q Versus WT Mice Treated With Saline

| | TNF- α (pg/mg) | IL-1 β (pg/mg) |
|--------------------|-----------------------|----------------------|
| WT+PBS | | |
| M (n=13) | 2.67±0.80 | 4.23±0.64 |
| F (n=11) | 3.34±1.24 | 3.45±1.42 |
| TNF1.6+PBS | | |
| M (n=11) | 286.03±33.0* | 232.3±44.7* |
| F (n=11) | 179.75±28.7* | 163.8±45.3* |
| TNF1.6+cV1q | | |
| M (n=6) | 1350.76±174.1†‡ | 104.15±22.7 |
| F (n=7) | 819.84±67.2† | 38.09±7.04 |

M indicates male; F, female; and PBS, phosphate-buffered saline.

*P<0.05 vs WT; †P<0.05 vs TNF1.6+PBS same sex; ‡P<0.05 vs female TNF1.6+cV1q. All comparisons by ANOVA.

Cardiac Cytokine Expression

At 12 weeks of age, male and female TNF1.6 mice showed significantly elevated myocardial levels of TNF- α and IL-1 β relative to sex-matched WT mice (Table). In the saline-treated WT and TNF1.6 categories, TNF- α and IL-1 β levels did not differ between the sexes. After treatment with cV1q, cardiac TNF- α levels significantly increased further in TNF1.6 mice, with the level detected in males significantly greater than that detected in females. Both male and female TNF1.6 mice treated with cV1q demonstrated significantly lower levels of IL-1 β than did sex-matched TNF1.6 mice treated with saline. Although apparently elevated relative to saline-treated WT mice, the level of myocardial IL-1 β in cV1q-treated TNF1.6 mice was not significantly different from that of saline-treated WT mice.

Discussion

Anti-TNF- α therapies have proved effective in the treatment of inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease.⁷⁻⁹ The elevation of circulating and cardiac levels of TNF- α in human heart failure^{1,2,11} and the recapitulation of many aspects of human heart failure in animal models of elevated TNF- α expression^{3,10} argue for a possible therapeutic role for anti-TNF- α therapies in heart failure management. Previous animal studies^{5,10} suggest that a fusion protein of soluble TNF receptor and IgG may limit biochemical changes underlying heart failure and elicit modest improvements in cardiac function. However, our prior study did not consider the profound differences in the sex-specific progression of heart failure in the TNF1.6 model.^{5,6} In the present study, we carefully considered the sex-related differences that occur in the age of onset and severity of heart failure in the TNF1.6 mouse while assessing the use of an anti-TNF- α antibody in treating or preventing heart failure progression.

cV1q is a monoclonal antibody with neutralizing activity against mouse TNF- α and contains rat IgD variable region domains expressed as a fusion protein with murine IgG2a constant domains. In some respects, this antibody resembles cA2 (infliximab), a partially humanized mouse mAb directed

against human TNF- α , which has been successfully used in the treatment of inflammatory diseases.^{8,9} Our previous studies used a recombinant adenovirus in a gene therapy approach to achieve expression of a fusion protein consisting of human soluble TNF receptor 1 and mouse IgG (sTNFR1-IgG).^{4,5} However, serum levels of the fusion protein markedly decreased with time, perhaps because of an immune response to the partially human sequences.

In the present study, we analyzed the sexes separately because treatment of male mice may represent a therapeutic treatment of overt failure, whereas treatment of female mice may resemble a therapy to limit progression. In female TNF1.6 mice, cV1q therapy significantly preserved basal fractional shortening and responses to β -adrenergic stimulation and partially limited cardiac dilation. These results are more striking than the results in our previous studies with sTNFR1-IgG therapy of TNF1.6 mice, which were performed only on female TNF1.6 mice after 2 weeks of therapy⁵ or on 12- or 48-week-old females after 6 weeks of therapy.⁴ Both prior studies demonstrated only modest cardiac dysfunction in 8- or 12-week-old female TNF1.6 mice (similar to this report) and either a significant reduction of cardiac dilation (LVSD)⁵ or a nonsignificant trend toward preservation of basal fractional shortening.^{4,5}

More remarkably, in male TNF1.6 mice in the present study, cV1q therapy significantly improved basal and β -adrenergic-stimulated cardiac function and reversed or limited progressive cardiac dilation. These novel results were not as apparent in previous studies using female TNF1.6 mice, which display only a modest cardiac dysfunction. This study of male TNF1.6 mice, which demonstrate a much more severe cardiac dysfunction, revealed a marked effect of TNF blockade on both limiting and reversing measures of heart failure and cardiac dilation.

Female mice treated with cV1q in the present study yielded a more notable response than previously observed with sTNFR1-IgG therapy. However, because our prior studies using sTNFR1-IgG therapy did not examine similarly aged male TNF1.6 mice, we can only suggest that cV1q provides greater benefit than sTNFR1-IgG treatment.

An interesting difference between the studies with adenovirus driving overexpression of the sTNFR1-IgG protein and treatment with cV1q antibody is the effect on expression of proinflammatory cytokines in the myocardium. Although both treatments appear to decrease the biological effects of TNF- α while increasing the level of cardiac immunodetectable TNF- α , probably through a stabilization of TNF- α protein and prolongation of half-life, the cV1q treatment did not fully normalize the level of immunodetectable IL-1 β , whereas the soluble TNFR1-IgG fusion protein did.⁵ These findings are consistent with the observation that the fusion protein decreased the amount of myocardial infiltrates,⁵ whereas cV1q therapy did not (data not shown). Whether this represents a fundamental difference in the effect of these 2 therapies is unclear.

Clinical trials in which either anti-TNF- α antibodies or soluble fusion proteins were used to treat inflammatory diseases have yielded mixed results in the attainment of therapeutic benefit. Although both approaches effectively

treat rheumatoid arthritis, anti-TNF- α antibody (cA2) is effective in the treatment of ulcerative colitis,⁸ whereas soluble TNF receptor-IgG fusion protein (etanercept) is not effective.¹² Although initial reports on the use of etanercept in human heart failure suggested modest beneficial effects,¹³ the clinical trial has recently been terminated for apparent lack of efficacy. However, it would not be without precedent if antibody blockade of TNF- α activity proved more effective than soluble receptor fusion protein in the treatment of congestive heart failure. This report demonstrates that, within the limits of an animal model of heart failure consequent to TNF- α overexpression, therapy with anti-TNF- α antibody can both improve and preserve cardiac function and limit cardiac dilation. Thus, the clinical evaluation of monoclonal anti-TNF therapy in patients with congestive heart failure may be warranted.

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